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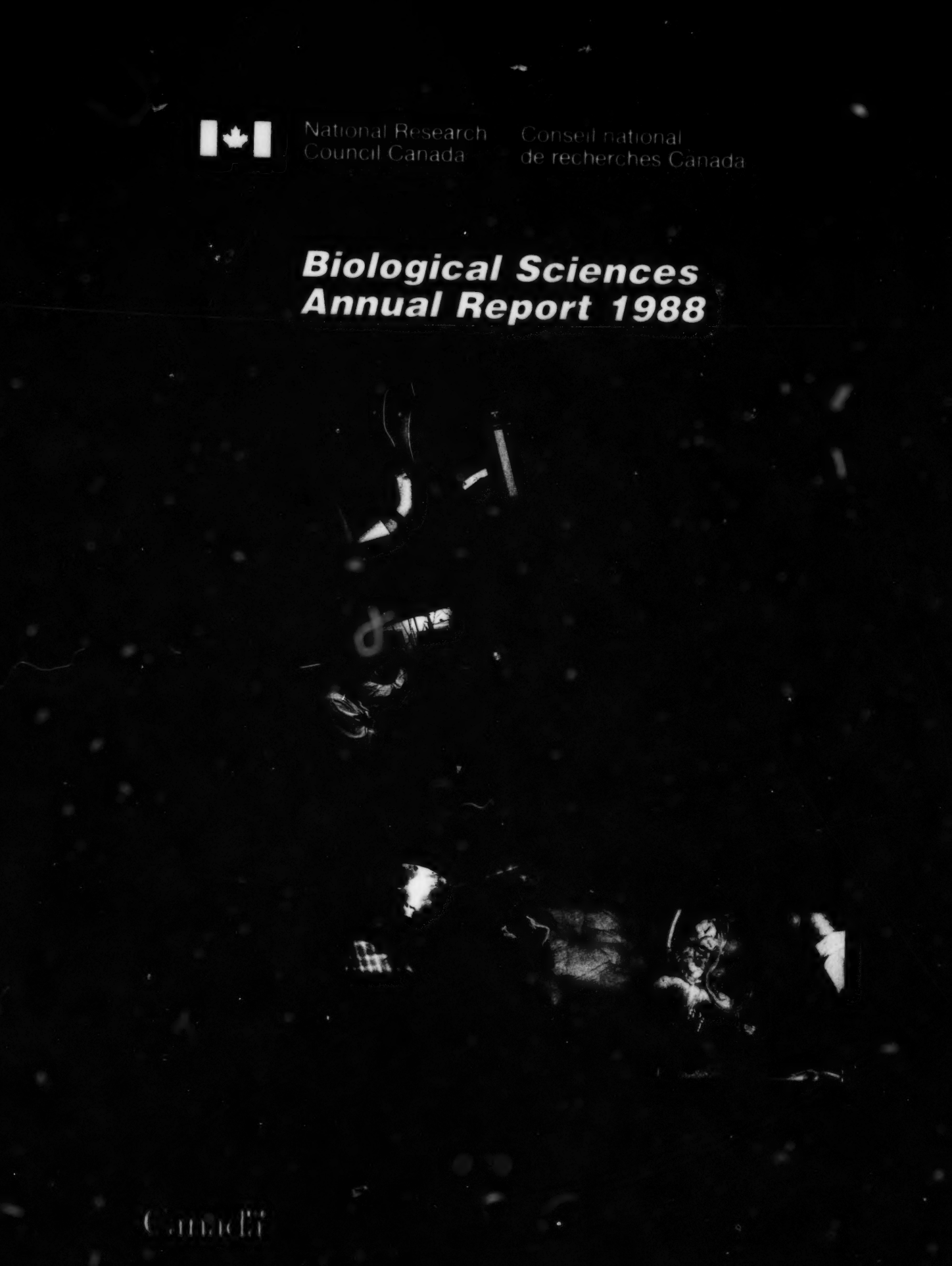
Biological Sciences Annual Report 1988



Biological Sciences Annual Report 1988

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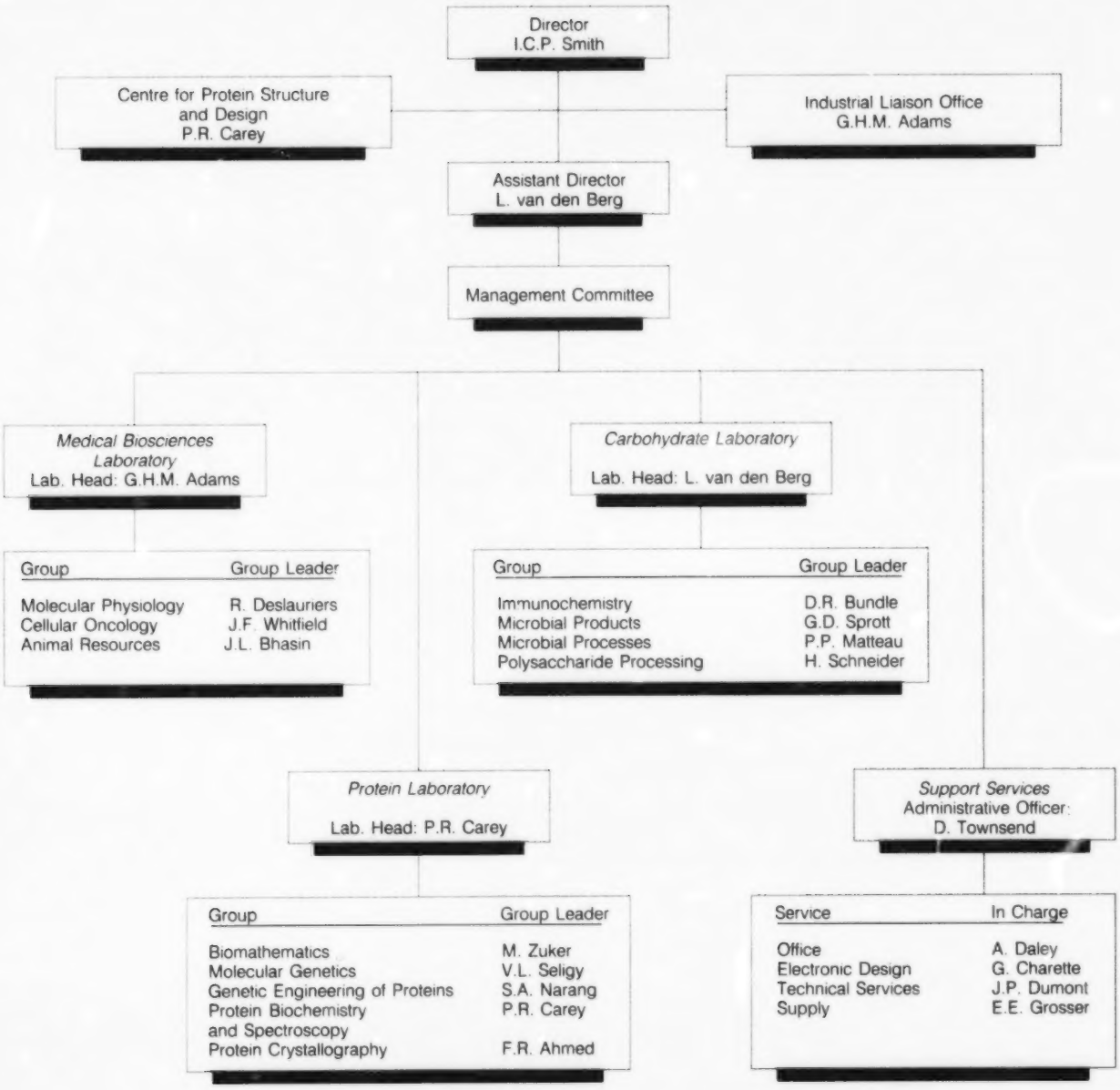
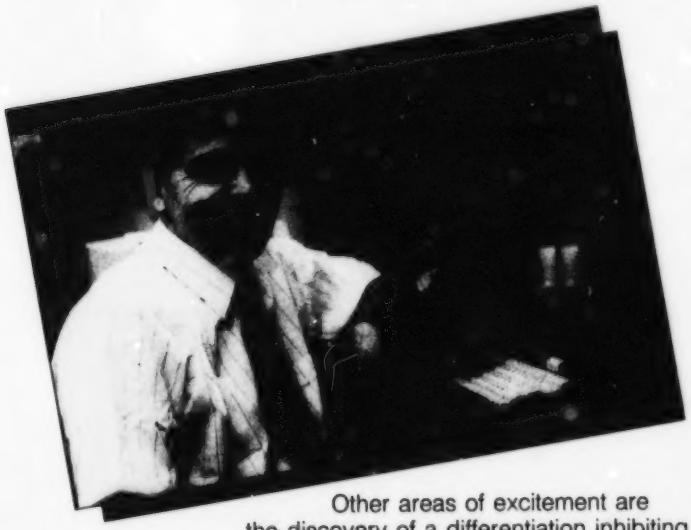
Organization: Division of Biological Sciences



Introduction

Scientific excellence in biological areas of industrial and social relevance is the mandate of the Division of Biological Sciences. The past year has seen some very significant strides.

Studies on protein structure and design, using X-ray crystallography and molecular modelling, have revealed the molecular structure of oncomodulin, a cancer marker discovered in the Division. Genetically engineered variants of this protein are also showing great promise as spectroscopic probes for minute quantitites of antibodies or DNA. The structure of an antibody fragment specific for the animal pathogen *Brucella abortus* is similarly being resolved and modified in antibody engineering studies. The light chain of the antibody has been cloned and expresed in active form in *E. coli*. Parathyroid hormone has been expressed in yeast and its structure is under analysis and modification in collaboration with industry.



Other areas of excitement are the discovery of a differentiation inhibiting factor of high potential utility in the diagnosis and therapy of cancer, the development of NMR techniques for non-invasive diagnosis of cancer and heart disease, and the revelation of crucial molecular features that direct the specificity of monoclonal antibodies. Interaction with industry, through the IRAP and Biotechnology Contribution Programmes of NRC, and the contracting-in process, has transferred technologies related to the production of flavours, pesticides, vaccines and diagnostics.

Interactions within NRC's Biotechnology Programme, with other NRC Divisions and with universities and industries continued to increase in intensity and scope. About 40% of the Division's efforts now involve external collaborations. At any time the Division's staff is complemented by a large number of guest researchers. The Centre for Protein Structure and Design is fully operational and attracting collaboration from industry and universities. Interactions in a wide variety of subjects have been augmented by the invited participation of the Division in Canada's Network of Centers of Excellence programme.

The Division was fortunate to upgrade its expertise and equipment in the area of protein structure determination. The recently approved expansion of our building which houses the medical NMR facilities will bring about a greater integration of our efforts in this area as well as improvement in the operating room and physiological facilities. The additional space will also allow greater interaction with our clinical and industrial partners.

A recent comprehensive peer review confirmed the appropriateness of most of our programmes and gave suggestions for improving some others. Their recommendations have been implemented to ensure a focussed and integrated research programme for excellence in selected areas and responsive to Canadian needs. This has been achieved through the continued dedication and enthusiasm of the Division's staff, who have contributed enormously in a year of considerable change.

Ian C.P. Smith
Director

Highlights of Divisional Activities

The objective of the Division of Biological Sciences is to do generic frontier research in specific areas of biotechnology that require a multidisciplinary approach, hold good potential for application, and are oriented towards industrial and economic development. Research is focussed in the following areas: molecular genetics, protein engineering, molecular medicine, molecular immunology, anaerobic microbiology, biological degradation of undesirable materials, and biological production of fine chemicals. The application of these research areas is highlighted in the following sections. Many of the studies involve collaboration with interested industries.

Molecular Genetics

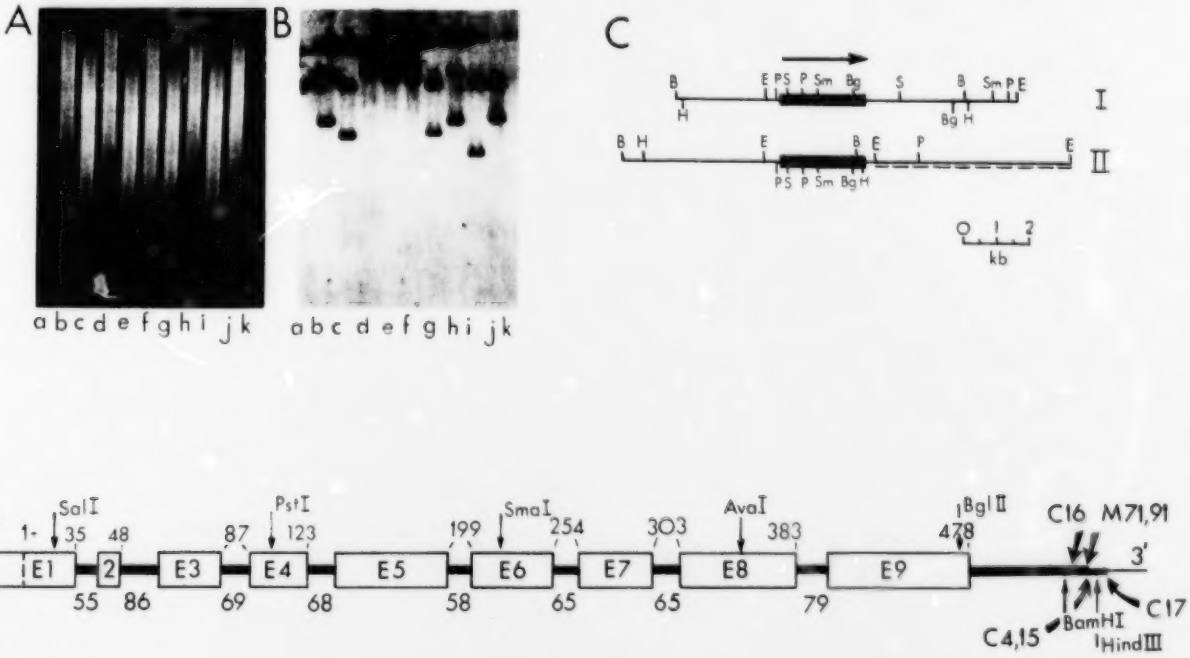
The knowledge gained in the genetics and molecular biology of both procaryotes and eucaryotes is being used to express foreign proteins in bacteria, yeasts, filamentous fungi, insect cells and mammalian cells. These systems, along with conventional and site-directed mutagenesis, are being used either to produce new strains for industrial processes or for carrying out protein engineering studies to design new and useful enzymes and hormones.

Examples of projects in this area are:

- Expression of human parathyroid hormone in *E. coli* and yeast
- Development of a Tipula iridescent virus-insect cell expression system for mammalian proteins
- Chemical synthesis and expression of genes coding for the Fab antibody fragment for a salmonella O-antigen in *E. coli*

Protein Engineering

The ability to clone and express proteins and to manipulate their genes to produce redesigned proteins with better industrial properties requires interdisciplinary teams of researchers for protein



The mapping of the gene coding for Taka-amylase A by the Molecular Genetics Group.

purification, characterization and modification. The development of efficient purification schemes for proteins of interest, as well as the expertise and instrumentation for determining their structure and biological activity, is essential to any protein engineering program.

X-ray crystallography plays a key role in protein engineering studies. The technique elucidates the detailed three-dimensional structure of macromolecules setting the stage for establishing structure-function relationships and ultimately designing new proteins with properties exactly suited to the desired industrial or medical application. Various techniques in protein spectroscopy are applied to problems involving protein structure-function correlation. Molecular modelling by interactive molecular graphics assists in the prediction of the properties of mutant structures and protein-ligand complexes. These studies are facilitated by sophisticated computer programs and databases.

Examples of projects dealing with protein engineering include:

- Structure of cysteine and serine protease with bound substrates
- Raman and CD spectral analysis of insecticidal proteins from *Bacillus thuringiensis* and the study of their degradation by sunlight
- Time-resolved fluorescence studies of the motion of protein segments containing aromatic amino acids in proteins such as oncomodulin and serine and cysteine proteases
- Structural and dynamical properties of parathyroid hormone

Molecular Medicine

Nuclear magnetic resonance (NMR) technology is increasingly being applied to problems of biomedical interest. In particular, NMR imaging is an emerging field which offers exciting prospects for the noninvasive diagnosis of a variety of human disorders including cancer, cardiovascular disease, and liver dysfunction. This powerful technology is also being used to test the effects of newly developed pharmaceutical agents on organ performance and metabolism.

Understanding the mechanisms which control the growth and differentiation of cells may be the key to unlocking the mysteries of cancer. Knowledge of how genes are regulated in cancer cells and the identification and isolation of unique products produced by cancer cells is pivotal to the development of pharmaceutical agents which can effectively combat this family of diseases.

Examples of projects of medical and pharmaceutical interest include:

- Metabolic and functional studies of preserved hearts destined for transplant
- Evaluating the effects of therapeutic drugs on myocardial function and metabolism
- Developing spectroscopic and imaging techniques for cancer diagnosis and management
- Developing imaging techniques for evaluating brain damage caused by stroke
- Exploring the therapeutic use of ADIF's (autocrine differentiation inhibiting factors) produced and secreted by hematopoietic cancer cells
- Identification of proteins which regulate the expression of genes involved in cancer development and metastasis

Molecular Immunology

Bacterial cell surface carbohydrate antigens are investigated for the development of new and better vaccines, diagnostics and targeting agents. The key is the determination of the exact structural and three-dimensional shape of the carbohydrates using advanced mass-spectroscopic and NMR techniques, as well as chemical synthesis. Immunological properties, including the production of antibodies, depend on the precise configuration of the antigens. Exciting new areas of endeavour are the analysis of antigen-antibody interactions and their combining sites, and the production of specific improved antibodies through genetic engineering.

Examples of projects:

- Determining the composition and three-dimensional structure of polysaccharide antigens of human and animal microbial pathogens and the development of diagnostics and vaccines
- Development of conjugate vaccines for human microbial pathogens
- Study of molecular recognition at antigen/antibody combining sites and the genetic engineering of improved antibodies

Anaerobic microbiology and degradation of undesirable materials

Pulp and paper industry wastes and chlorinated phenolic compounds are the focus for developing anaerobic processes for waste treatment. The approach includes microbiological studies as well as bench-scale applied studies. These studies also include the dynamic and steady-state modelling of all the pertinent information to assist understanding of the complex phenomena involved and to help scale-up of results to industrial scale reactors.

Examples of projects in this area are:

- Adaptation of advanced anaerobic reactors to pulp and paper wastes
- Anaerobic microbial breakdown of resin acids and chlorinated phenolic compounds
- Development, study, and modelling of advanced anaerobic reactors

The anaerobic waste treatment laboratory of the Microbial Processes Group at Rideau Falls.

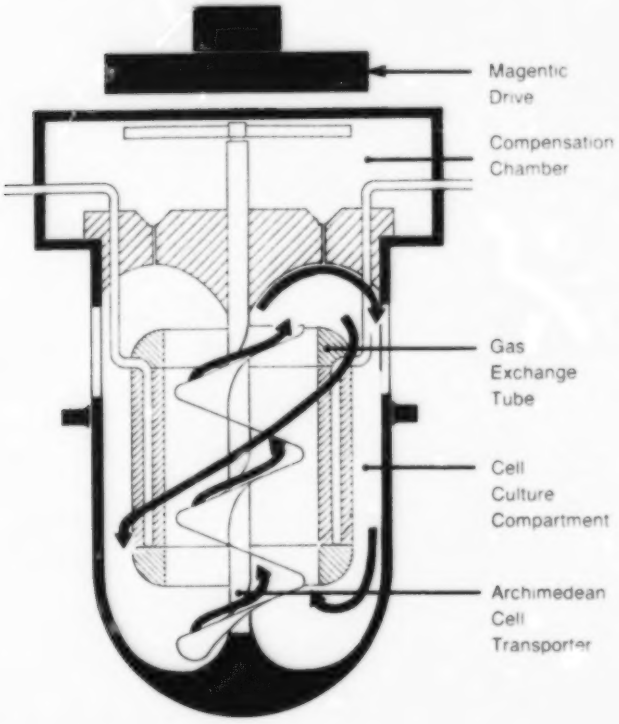
Biological Production of Fine Chemicals

Flavours, biopesticides and enzymes are some of the specialty chemicals for which microbiological and enzymatic processes are being developed for industrial use. Studies are based on the broad capabilities of microorganisms and enzymes to effect novel conversions, particularly when placed in a unique environment such as non-aqueous solvent systems. Strong industrial collaborations, combined with a fermentation facility which allows production in larger quantities, allow bridging the gap between basic science and commercial exploitation.

Examples of projects:

- Use of lipase/esterase enzyme systems in non-aqueous media for the production of flavour compounds
- Development of fermentation systems and immobilization technologies for the production of biopesticides

Sketch of an animal cell bioreactor recently developed by the Microbial Products Group.



Industrial Liaison Office

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G.H.M. Adams
Assistant Industrial Liaison Officers:
Protein Laboratory:
S. Hasnain
Medical Biosciences Laboratory:
J.P. Durkin
Carbohydrate Laboratory:
D.W. Armstrong

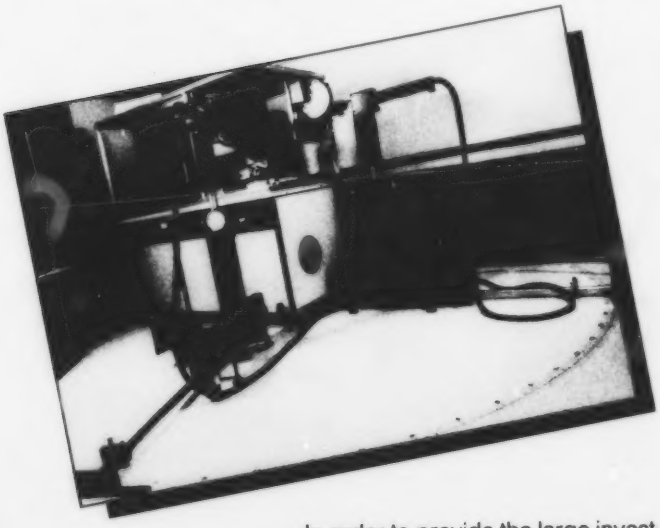
To facilitate interaction between its research groups and appropriate industrial partners, the Division of Biological Sciences maintains an Industrial Liaison Office. The role of this office is to increase the level of awareness in the Division of the needs and interests of Canadian industry in areas related to biotechnology, to inform industries of the technical opportunities offered by interaction with scientific groups in the Division, and to foster and to look after all the mechanisms of interactions and technology transfer to industry. This has involved many visits to industry and arrangement of visits from industry to the Division. It has also involved close interactions with various groups within NRC: the Industrial Programs Office at the Biotechnology Research Institute (BRI), the Field and Laboratory Networks of the Industrial Research Assistance Program (IRAP), the Marketing Group, Industrial Liaison Officers of other divisions, and the Office of Legal Services.

The Division is involved in many formal joint research projects with industrial clients with financial and intellectual input from both parties. In many cases, the companies also receive support from the Biotechnology Contribution Program or from IRAP. These joint projects involved over 1 million dollars of research expenditures by about 15 clients. They are in the areas of natural flavours, biopesticides, anaerobic waste treatment, protein purification, immobilization systems, development of bioreactors, antibody research and drug testing. Company personnel often work directly with NRC scientists at the Division of Biological Sciences. In 1988, there were 18 industrial people working in the Division.

During the last year, industrial liaison officers have been appointed from each of the three Laboratories: Carbohydrate, Medical Biosciences, and Protein. This has greatly increased our industrial contacts and has insured a closer involvement of the Laboratories in the industrial activity. Greater effort will be directed to promoting the unique facilities in the Division and their use by outside clients as well as on the development of more collaborative projects with industries based on the expertise and research focus in each of the Laboratories.

Centre for Protein Structure and Design

NRC's Centre for Protein Structure and Design provides and coordinates the high technology and expensive techniques which are needed to determine protein structure and properties. Thus the expertise at the Centre consists of protein crystallography, protein NMR, powerful computational facilities and spectroscopic techniques such as circular dichroism (CD), fluorescence and Raman. The Centre is not a Protein Engineering Centre as such but it does provide the essential biophysical support needed for protein engineering programmes and it can also, of course, characterize other novel and interesting proteins which are not part of protein engineering studies.



X-ray diffractometer with area detector, operated by the Protein Crystallography Group as part of the Centre for Protein Structure and Design.

In order to provide the large investment in personnel and capital required by a state of the art Centre, resources have been allocated from both the Biotechnology Research Institute in Montréal and the Division of Biological Sciences in Ottawa. Thus, the instrumentation for protein NMR is located in Montréal. The Division's contribution to the Centre consists of its protein crystallography, protein biochemistry and spectroscopy and biomolecular modelling groups. These sections moved to the new extension to the Division's building at Montreal Road (M-54). The move to M-54 has been accompanied by the installation of new equipment to ensure that each technology contributing to the Protein Centre is up-to-date.

In order to remain at the cutting edge of research each group at the Centre spends a large fraction of its time engaged in fundamental studies. However, one important aspect of the Centre is that it will provide a nucleation point for scientists from Canadian industry and universities to interact with NRC staff. Some of these interactions may be simply of a service nature while others may involve major collaborations on problems of mutual interest. As such collaborations get underway provision will be made for industrial or academic scientists to stay at the Centre for long or short periods of time.

Research Facilities

— Fermentation facility ('C' level containment) - 0.5 L to 250 L fermentors. Tangential flow membrane harvest equipment as well as high capacity continuous flow centrifuges. Large volume 18 megohm water generation module.

— Pathogen containment and fermentation facility - 28 L and 75 L fermentors with cell harvesting equipment for culturing pathogenic organisms.

— Culture collection - in-house collection of more than 2500 species of bacteria, yeast and fungi, most of them available on request.

— Animal facility - specific pathogen free and gnotobiotic mice, rats and rabbits, nude mice, and conventional animals for research purposes.

— NMR - 500, 360 (2), 200 MHz high resolution instruments; 300, 200 MHz broad line instrument; 200 MHz whole body spectrometer for spectroscopy and imaging.

— ESR - 9 GHz instrument.

— X-ray diffractometer with area detector.

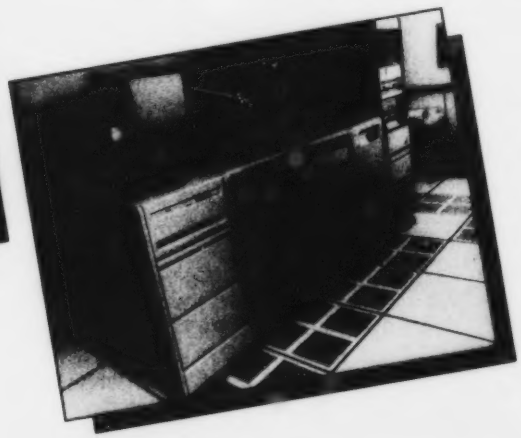
— Raman spectroscopy - lasers and spectrographs for Raman spectroscopy in the visible and uv regions.

— Steady state and laser-based time resolved fluorescence instrumentation.

— Circular dichroism spectroscopy (uv and visible) - Jasco J600, thermostatted.

— Computers - Vax 11/750; high speed links to IBM mainframe and Vax 780 containing protein and DNA data bases and protein analysis routines.

Fermentation facility operated by the Microbial Products Group.



Computer room managed by the Biomolecular Modelling Group.

- Molecular graphics system - Evans and Sutherland, and Silicon Graphics (IRIS 40-70 GT).
- Amino acid sequenator - (2) Applied Biosystem, gas phase; (2) Beckman 890, liquid phase.
- Amino acid analyzer - Durrum D-500.

Amino acid sequenator in the Molecular Genetics Group.

- Peptide synthesizer - BioSearch Sam II.
- DNA synthesizers - (2) BioSystems.
- C-level rDNA containment facility.
- Cation biofluorometer - Spex CM system - specificity for intracellular Ca^2.
- Stereotaxic apparatus and microinjection capability.
- Cytofluorographic facility.
- Mammalian cell culturing laboratory.
- Hybridoma production laboratory.

Protein Laboratory

Head: P.R. Carey

Biomolecular Modelling
Group Leader: M. Zuker

Professional Staff:
R. Somorjai, M. Zuker
NSERC Fellow:
M. Sylvain
Visiting scientist:
M. Phipps (University of Ottawa)
Technical staff:
L. Bramall, J. Ridgeway,
Co-op student:
E. Nelson

The objective of the Biomolecular Modelling Group is to develop mathematical algorithms to analyze and compare the information contained in biotechnology databases and to simulate

the behavior of the nucleic acids and proteins stored in them. These databases comprise genetic and physical mapping data, DNA and protein sequence data, and protein structure data. The group currently has three areas of interest. The first one is the development of pattern recognition algorithms to analyze nucleic acid and protein sequences. Such methods can be used to predict intron/exon boundaries or protein coding regions in DNA. With protein sequences, one can predict a secondary structure assignment for individual residues or else predict both the functional and structure class of the entire protein. Another area is the development of algorithms for pairwise or multiple sequence comparisons. These algorithms can be used to establish whether or not two proteins are related. When homology is not in doubt, alignment can be used to predict structural relations between proteins. The last area is the deduction of higher order structure from sequence data, both for RNA and proteins, and the development of algorithms to automatically compare and align protein structures in three dimensions.

The RNA folding program which predicts suboptimal structures was refined, and related programs were also written. The resulting algorithm now gives extra stability to designated tetra-loops (hairpin loops containing four unpaired bases), and will also permit a simulation of the folding of the entire HIV virus. A computer company has expressed interest in this area.

A quantum mechanical method has been applied to an ab initio folding model for the prediction of protein structures. It avoids the problem of becoming trapped in local energy minima by using a probabilistic model which computes expected coordinates. An improved Monte Carlo algorithm has been developed for use in protein folding and energy minimization algorithms. It keeps track of which random motions produce the greatest results, and concentrates on these. Another computer company is interested in incorporating this idea into its quantum chemistry software.

A sequence analysis algorithm was created which predicts the structural class of a protein from its amino acid composition. Five structural classes are used, based on different combinations of alpha helix and beta sheet content.

An algorithm for aligning protein structures in three dimensions was also developed, and a link has been established with a group at the NIH in a search for new and improved scoring matrices for protein sequence comparisons. The idea is to develop a matrix which works best for distantly related proteins. This has been coupled with work here on expanding the notion of a gap and on predicting suboptimal alignments. Two proteins which might seem totally unrelated at the sequence level using standard alignment procedures can sometimes be shown to be related. Calibration of this method is being done on groups of proteins with known three dimensional structures. An algorithm which uses hierarchical clustering to align groups of nucleic acid or protein sequences was also created. It is being calibrated using a group of 5 xylanases and 16 cysteine proteases. An algorithm to further refine the multiple alignment is under development.

The group maintains a VAX 11/750 computer for the use of the Protein Structure and Design Centre. We have high speed links to an IBM 3090 computer with a vector coprocessor and a local area network linking workstations with the VAX and IBM computers. An LSI minicomputer, running a collection of our own analysis programs, is maintained for the use of the Molecular Genetics Section. The AMBER program for protein structure dynamics and energy minimization has recently been adapted for use on the IBM computer. We have close ties with a group at the Canadian Institute for Scientific and Technical Information (CISTI) that is responsible for maintaining the biotechnology databases outlined above. The information contained in these scientific numeric databases and analysis software are available for use across Canada.

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Nonlinear methods for discrimination and their application to classification of protein structures.
J. Theor. Biol. 130: 461-468, 1988.

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Distinctive properties of signal sequences from bacterial lipoproteins.
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Deslauriers, R., Somorjai, R.L., Geoffrion, Y., Kroft, T., Smith, I.C.P. and Saunders, J.K.
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NMR in Biomedicine, 1(1): 32-43, 1988.

Genetic Engineering of Proteins
Group Leader: S.A. Narang

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Guest Scientist:
S. Mandal
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G. Dubuc, J. Michniewicz
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C. Chaudhary, A. Tonary
Summer Students:
D. Horvat

The main goal of this group is to design and produce novel proteins of potential medical and industrial importance by synthetic genes and using the recombinant DNA approach. Our research efforts have dealt with lysosymes and xylanases, but are recently focussing especially on antibody fragments.

Lysozymes are an important class of enzymes on which extensive work has been done in the past 30 years. The accumulated knowledge makes T_4-lysozyme an excellent model for protein engineering. We have synthesized and expressed the gene in *Escherichia coli*. The X-ray crystal structure of the synthetic T_4-lysozyme has been solved and compared with the wild type. A minor difference was found near the C-terminal. Its active site has been unambiguously established by mutating the active-site amino acid residues. A mutant T_4-lysozyme (Asp11-Glu20) has been purified for further X-ray crystallization studies.

A xylanase gene from *Bacillus circulans* has been cloned and expressed in *E. coli*. Xylanase from this recombinant construct has been isolated, purified and crystallized to determine its structure. The active site residues have been identified by site-directed mutagenesis. Thermostable xylanase has also been cloned and expressed in *E. coli*.

More recently our efforts have been focussed on genes coding for light and heavy chains of the Fab (antibody) fragment specific for *Salmonella* O-antigen as part of a broader approach to antibody engineering. These genes have been synthesized in *E. coli* and have been fully characterized for their DNA sequences. The light chain gene has been successfully expressed in *E. coli* under the lac-promoter. The work on its characterization is done in close collaboration with the Immunochemistry group of the Division.

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Crystal structure of T_4-lysosyme generated from synthetic coding DNA expressed in *Escherichia coli*.
Protein Engineering, Vol. 2(4); 277-282, 1988.

Molecular Genetics
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Undergraduate Students:
Y. Adcharamoorthy, S. Bottega, C. Gervais, K. McDermit (Carleton), J. Pelletier (Ottawa), P. Shwed

This multidisciplinary group studies gene and protein primary structure, expression and regulation. Fundamental and applied problems are tackled in collaboration with other groups in NRC, government, industry and universities. Focus is on three areas:

characterization and production of enzymes; characterization and production of proteins that regulate the growth, development or differentiation of cells; and the development of rDNA modified organisms.

The group has characterized several glycosidases and/or their genes from bacteria or lower eukaroyotes, including mutants of the *Bacillus subtilus* endocellulase, expressed in *E. coli*. These studies have shown that the first 162 amino acids of the C-terminus are not needed, but that the removal of 16 more deactivates the enzyme. The 308 amino acid core shows the highest identity to other bacterial cellulases, including that of the plant pathogen, *Erwinia chrysanthemia*. The amino-acid sequence of two fungal xylanases have been characterized and compared to other xylanases whose sequences were known, and the active site was tested by site-directed mutagenesis. Other glycosidases studied include an amylolytic enzyme and "Taka-amylase A".

Most of the proteins that modulate expression of specific genes during switches in cell metabolism and development have yet to be discovered. Purification and physical characterization of one such protein, PTH are in progress. The group continues to focus on this exciting area of research, capitalizing on existing expertise in genetics of lower organisms and growing expertise in animal cell genetics. For this the genetics and expression of a broad host range, double stranded, cytoplasmic DNA Iridovirus is also being studied. Components of a gene from this virus and other genes should be useful in developing novel expression vectors for comparison with existing systems and in the production of animal proteins. Present efforts include setting up small-scale cell culture systems for study of animal genes.

The Group's recognised expertise in amino acid sequencing is used extensively in NRC and outside. The amino acid sequence of the putative Dutch elm disease toxin has been obtained and the structure is under study. Partial or full amino acid

sequences of three murine monoclonal antibodies, several proteases, and two plant lectins have also been obtained.

Anand, N.N., Stephen, E.R. and Narang, S.A.
Mutation of active site residues in synthetic T_4-lysosyme gene and their effect on lytic activity.
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Protein Biochemistry and Spectroscopy
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A major focus for this Group is the provision of optical spectroscopic tools needed for protein characterisation at NRC's Centre for Protein Structure and Design. The Group has expertise in fluorescence, Raman and resonance Raman spectroscopies and has recently set up a facility in circular dichroism spectroscopy. The latter is the responsibility of Dr. G. Willick who, with his research team, joined Protein Biochemistry and Spectroscopy in the Spring of 1988. In addition to research in protein spectroscopy there are three projects involving protein purification and characterisation, each of which involves extensive interactions with other groups at NRC, Canadian universities and industries. Two projects involve mammalian systems in the form of cysteine protease enzymes and human parathyroid hormone (PTH). The third project concerns the environmentally safe insecticidal proteins from *Bacillus thuringiensis*. Efforts in the first two projects have focussed on purifying milligram amounts of human cathepsin B and cloned PTH for biochemical and physical characterisation. For B.t. proteins, large amounts of material have been purified and subjected to extensive biophysical and protein chemical examination.

Efforts in the Raman spectroscopy laboratory center around two objectives. One is to use normal Raman spectroscopy as a technique for providing information on protein secondary structure and the properties of amino-acid side chains. This has been used effectively to characterise the proteins from *B. thuringiensis*, for example. The novel technique of Fourier Transform Raman spectroscopy, employing an infrared laser and FT detection is being investigated as a means of overcoming 'fluorescence interference' and making Raman spectroscopy into a facile routine technique for characterising proteins, either native or produced by protein engineering.

The other objective of the Raman laboratory is to obtain detailed molecular information on enzyme-substrate complexes by resonance Raman spectroscopy. A notable recent advance is the use of low temperature (77°K) resonance Raman measurements on enzyme-substrate complexes to characterise the effect of protein thermal energy on catalytic events in the active site. A further area of progress concerns the discovery that the C=O group undergoing catalytic attack in the active site can be monitored via its resonance Raman signature and that the latter provides a direct correlation between structure and reactivity.

In the fluorescence laboratory efforts to acquire and analyse high quality time resolved fluorescence data are at an advanced stage. Multi-exponential fluorescence decay curves can now be analyzed with confidence and each component in the decay has been assigned to a separate conformational entity in the protein under study. Thus, the technique provides information on protein dynamics and on the existence of the number of interconverting conformational states.

The time-resolved fluorescence approach can be applied with the most rigour to proteins containing a single tryptophan residue. The calcium binding proteins parvalbumin and oncomodulin have provided paradigms for time-resolved studies. Analysis of the results for parvalbumin demonstrated the existence of three exponentials in the decay curve. Studies on oncomodulin have been assisted by the production of site-specific mutants (in collaboration with J. MacManus, DBS and D. Banville, BRI) and efforts are underway to redesign oncomodulin by protein engineering to enhance the molecule's metal binding capabilities.

Fluorescence analyses of enzymes and enzyme substrate-complexes, involving the subtilisin family, have begun with a view to correlating enzymatic activity with the dynamic properties revealed by fluorescence analysis.

In the area of protein purification and characterisation the Group has concentrated on three projects. The Group's long term expertise on cysteine proteases has been focussed on cathepsins. These include mammalian cysteine proteases which are targets for novel drugs aimed at controlling disease states such as muscular dystrophy. The groups efforts center on defining the specificities of cysteinyl cathepsins by determining where they cleave protein substrates, and on detailed kinetic and structural definition of the enzymes, with the eventual goal of X-ray crystallographic analysis.

The second protein system concerns the insecticidal family of proteins produced by *B. thuringensis*. Work has concentrated on producing large amounts of protein for biochemical and biophysical examination. Progress has been accelerated in the past year by the determination of HPLC protocols for isolating large quantities of very pure B.t. toxin. Detailed analysis of the cause of sunlight inactivation of B.t. proteins has continued.

The third and expanding activity involves a collaboration between the Group and the molecular Genetics section on the cloning, expression and purification of the calcium regulating parathyroid hormone. Current purification efforts are concentrating on material expressed from yeast and concurrently on improving the level of expression.

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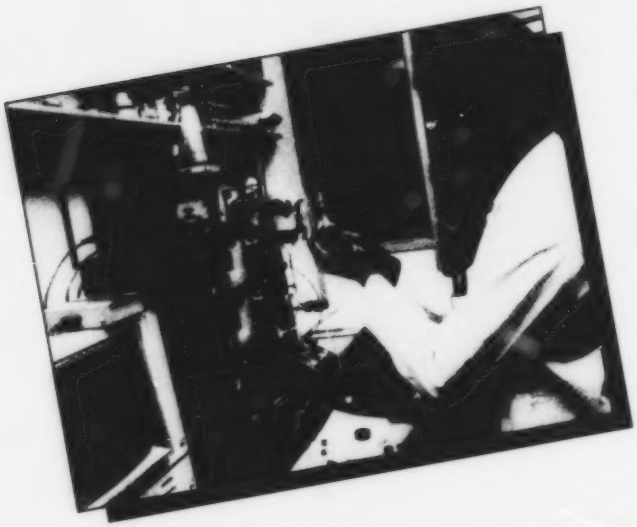

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Protein Crystallography
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Solving the three-dimensional structures of proteins by X-ray crystallography is the primary objective of this group. The results of these analyses provide a sound basis for understanding protein interactions, for the design and engineering of certain function-specific proteins, and for drug design. As part of the Centre for Protein Structure and Design, the group was funded for major equipment replacement and has installed a high-power rotating-anode X-ray generator, a microVax- 3500 computer, a Huber goniostat, and a multiwire area detector system for fast, efficient data collection.

Other major equipment includes a Nonius CAD-4 diffractometer and two interactive high-resolution computer graphics systems.

A major highlight this year has been the determination of the crystal structure for oncomodulin, the calcium-binding protein found in about 90% of human and animal tumours but not in "normal" cells except placental cytotrophoblast cells. It is hoped that comparison of this structure with those of related calcium-binding regulatory proteins will lead to satisfactory answers to some basic questions about cancer cells. The Cellular Oncology Group provided the material; the analysis was performed with 5772 reflections in the 1.8-6.0 °A resolution range.

In collaboration with the Protein Biochemistry and Spectroscopy Group and groups at the BRI, we are studying the interaction of papain and related cysteine proteases with substrates and inhibitors. A publication describing the crystal structure of a papain-E64 complex is now in press, and the structure determination of a papain-dodecamer complex is well underway. We have been able to grow minute crystals of cathepsin B, and are continuing trials to improve on their size and quality.

There has been considerable progress on the structure analyses of an Fab specific to *Brucella abortus* (produced by the Immuno-chemistry Group) and of yellow-fin tuna myoglobin, which is being studied in collaboration with the Protein Biochemistry and Spectroscopy Group.

In addition to the initial xylanase material which was produced by Forintek Canada from *Trichoderma harzianum*, we now have a xylanase from *Thermoascus aurantiacus*, and another one from *Bacillus circulans* produced by the Polysaccharide Processing Group. All have been crystallized and attempts at producing heavy-atom derivatives are in progress.

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Medical Biosciences Laboratory

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Molecular Physiology
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The objectives of the Molecular Physiology Group are to develop and use non-invasive techniques to study problems of biomedical importance. Part of the group's activities are related to technique development, both spectroscopic and physiological. A major part of the work in the group involves application of these non-invasive spectroscopic and physiological techniques to solving specific problems of biomedical importance. The projects which are undertaken may be developed "in-house" or arise from requests or collaborative work with universities, hospitals or industry.

During 1988, as a result of the amalgamation of the Molecular Biophysics and Animal Physiology Groups, a considerable number of projects have been terminated or re-oriented.

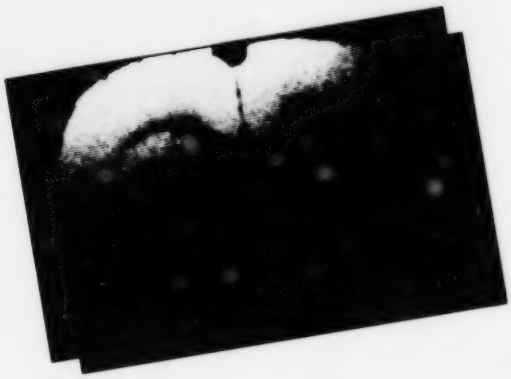
Work on model membrane systems is continuing with particular emphasis on understanding the motional characteristics, conformations and surface orientations of membrane surface carbohydrates and how these properties influence their interactions.

In NMR technology, a major fraction of our resources has been dedicated to building the probes required for studies of perfused organs and to the development of microimaging devices.

In our studies of hearts, we have developed the capacity to perfuse pig hearts in the NMR spectrometer. These studies are intended to complement our work on preservation of human cardiac tissue which is currently modeled using human atrial appendages. The perfused pig heart should provide a model in which functional parameters can be monitored following preservation. We have also developed protocols to evaluate the effects of drugs on perfused rat hearts for use by the pharmaceutical industry.

The work on brain has made use of our unique microimaging capabilities to evaluate the effects of two different models of stroke on specific structures within the brain of the rat. We have also been evaluating the effects of different therapeutic modalities on brain as well as following the time course of changes induced in brain structures following a stroke.

Work on cancer in the Molecular Physiology Group can be divided along two main lines: one dealing with diagnosis and evaluation of malignancy, metastasis and staging of human cancers using blood and other tissue samples, the other utilizing animal models of cancer as a basis for the development of spectroscopic and imaging techniques with which to evaluate human cancers.



NMR image of a rat brain.

We have been using liver both as a test system for evaluating non-invasive techniques of studying metabolism and to evaluate the effects of alcoholism on the energetic status of the liver. In the latter case we have found a good correlation between NMR and histological parameters.

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Magn. Res. Medicine, 7, 463-471, 1988.

Cellular Oncology
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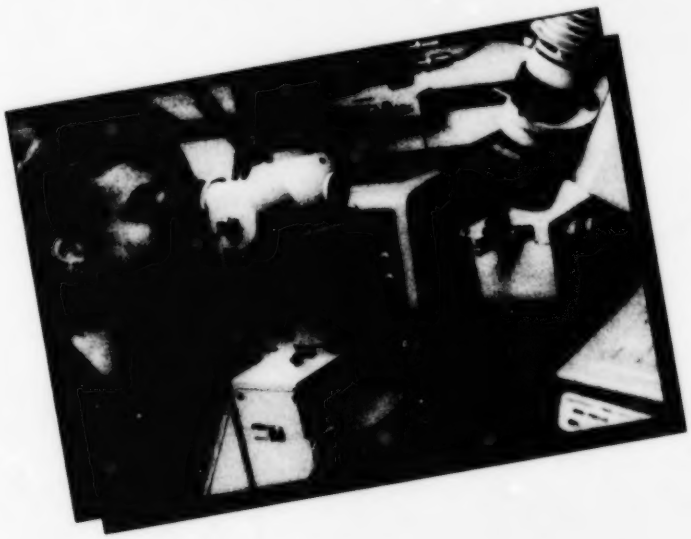
The general mandate of the Cellular Oncology Group is to determine how normal animal cells switch from a non-proliferative to a proliferative mode and **vice versa** and what goes wrong with these switching mechanisms to produce cancer. During the last 20 years we have identified Ca^{2+}, Ca^{2+}-binding proteins, Ca^{2}-dependent "signalling" protein kinases and cyclic AMP and cyclic AMP-dependent protein kinases as principal participants in the events leading to chromosome replication. The evidence indicates that either the disturbance of or the loss of the need for the membrane based Ca^{2+}-dependent signalling mechanism is at least a major part of the dysregulation and ultimate deregulation of cancer cell proliferation.



To understand the dysregulation or deregulation of cancer cell proliferation, we must understand the regulation of normal cell proliferation. Since the rat liver is still the only experimental model in which a very large, accessible mass of non-proliferating, unquestionably normal cells can be stimulated to switch synchronously and temporarily into a proliferative mode, we are committing considerable effort to finding out how these cells use Ca^{2+} and cyclic AMP signals to control the level and activity of the key, rate limiting replication enzyme-ribonucleotide reductase.

A second major effort, a Franco-Canadian joint venture, is to characterize and exploit a newly discovered family of autocrine differentiation-inhibiting proteins which are produced by erythroid and myeloid leukemia cells. These proteins lock the producer tumor cells into an active proliferative state by preventing differentiation.

The signals that switch on proliferation are also being revealed by exploiting new methods for measuring internal Ca^{2+} levels and membrane-associated protein kinase C activity. In particular, we are using them to find out

how two viral oncogene products (the avian sarcoma virus' v-src pp60 tyr protein kinase, Kirsten sarcoma virus' p21 RAS protein) signal quiescent cells to start proliferating the first step on the road to cancer. These model systems are ideal for searching for proteins that are specifically related to neoplastic transformation and that could be exploited commercially for cancer diagnosis.

Many years ago our exploration of the universally disturbed role of Ca^{2+} in the control of cancer cell proliferation led to the discovery of a new Ca^{2+}-binding protein, oncomodulin. This protein appears in 94% of all adult human or rodent cancers but never in normal adult tissues. The only "normal" cells that produce oncomodulin are the highly invasive, destructive, quasi-metastatic cells of the embryonic cytotrophoblast, but not the moving, shaping, proliferating cells of the embryo itself. This suggests that oncomodulin is part of, or a byproduct of, an invading/spreading mechanism that is specifically designed to serve some function in the early embryo's probing, pioneering, placenta-forming cytotrophoblast cells. Obviously, when we find out what oncomodulin does for cytotrophoblast cells, we may learn why cancer cells start making it again in the adult animal.

We are also characterising the changes in chromatin structure which are responsible for the major alterations in gene expression that accompany proliferation, differentiation and carcinogenesis.

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The Journal of Biological Chemistry, 263(3): 3005-3011, 1988.

Professional Staff:
J.L. Bhasin
Technical Staff:
L. Dvarionas
Animal Care Staff:
J. Daoust, M. Cleroux, P. Dashney, J. Lapierre, M. Levert, M. Foster

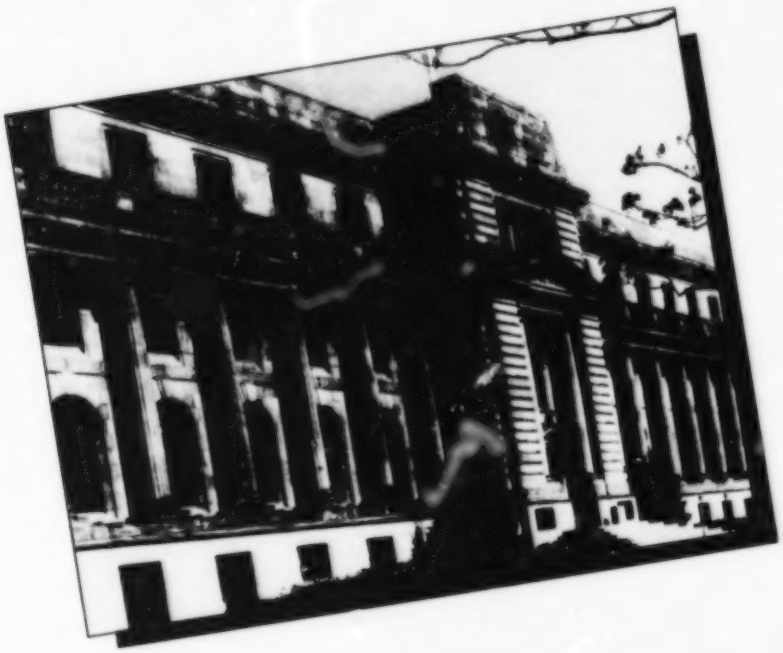
The main objective of the group is to provide disease free laboratory animals for research in the Divisions of Biological Sciences, Chemistry and Electrical Engineering of NRC. Animals are also provided to other institutions who have a specific need for well defined animals raised in our facility.

Presently animals are supplied to a total of 35 scientists in this Division, 5 in Electrical Engineering and 5 in the University of Ottawa. Of the animals cared for and provided, 75% represent specialized strains of rats and mice raised in our facility. We have well established colonies of germ free and pathogen free Sprague Dawley rats, and athymic mice and we have recently succeeded in establishing a gnotobiotic rabbit colony. The group also maintains tumor cell lines in germ free and SPF inbred nude mice and prepares polyclonal and monoclonal antibodies against microbial, viral and synthetic antigens in laboratory animals.

A secondary important objective is research on selected pathogenic organisms of veterinary importance in order to develop veterinary diagnostics and vaccines. We also assist the poultry and agricultural industry, government and other agencies in Canada, in ascertaining the epidemiological status of acute infectious diseases of poultry and livestock. Current efforts have aided in establishing the prevalence of *Pasteurella multocida* serotypes causing fowl cholera in turkeys and chickens and have helped to identify situations where vaccination of poultry or livestock with the currently available vaccines would be feasible if newly recognised serotypes would be incorporated in bacterins. Analysis of major antigens of the organisms responsible for serotype specificity and immunospecificity has shown that each serotype is antigenically complex and serologically as well as immunologically distinct.

Carbohydrate Laboratory

Head: L. van den Berg



Immunochemistry
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Guest Scientists:
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Technical Staff:
J. Christ, F. Cooper, E. Eichler, M.A. Gidney, B. Harrisson, R.A.Z. Johnston, R. Leitch, L. MacLean, S. Raymond, B. Sinnott
Summer Students:
S. Germain, M. Kelly

Complex carbohydrate antigens of bacterial and mammalian cell surfaces and their interactions with protein receptors such as antibodies and lectins are the major interest of the Immunochemistry Group. Molecular recognition especially the specific binding of oligosaccharides by monoclonal antibodies forms the basis of an expanded research effort. A multidisciplinary approach that involves the Protein Crystallography and Genetic Engineering of Proteins Group is directed toward crystallography of selected Fab fragments derived from monoclonal antibodies and antibody engineering of these combining sites. The objective of the work is to understand in atomic detail the selectivity and exquisite differentiation of cell surface oligosaccharides by protein binding sites, basic knowledge that is regarded as a first step toward the modification and rational design of enzymes and carbohydrate receptors.

David Bundle

The diverse expertise and physical resources of the section in areas of immunology, biochemistry, analytical chemistry and synthesis are also used to develop novel solutions to longstanding problems in clinical and veterinary microbiology. Emphasis is placed on the interpretation of biological activity in terms of the chemical properties and three dimensional structures of the antigens and the corresponding antibodies. These are useful in diagnosis and offer protection from human and veterinary diseases. This approach provides a scientific basis for the development of vaccines and diagnostics, and results from several socially and economically significant projects have been exploited by Canadian industries.

An integrated approach to research is made possible by the extensive and diversified facilities of the group which include a level E microbial containment facility, a monoclonal antibody production unit and an analytical laboratory providing facilites for high resolution nuclear magnetic resonance spectroscopy and combined gas chromatography–mass spectroscopy. The pathogen containment unit is equipped with 28L and 75L fermenters that provide for large scale production of bacterial cells. High resolution ^{1}H and ^{13}C NMR spectroscopy performed on Bruker AM200 and AM500 instruments

and mass spectroscopy are the principal methods used to deduce the complex structures of bacterial antigens. A micro-Vax 3500 is used to perform off-line processing of NMR data and for computer assisted modeling of carbohydrate antigens by a combination of potential energy calculations and NMR measurements.

Highlights of recent progress by the group are to be seen in the human health field where emphasis on a new generation of sub-unit vaccines developed by chemical modification of polysaccharides has led to a product that overcomes many of the immunological

Larger scale production of monoclonal antibodies.

deficiencies associated with present day vaccines. Meningococcal meningitis is one of the principal infections against which vaccines of this type are most effective, and the group's research has also resulted in significant progress toward vaccines for *Streptococcal* infections. In addition to offering solutions to practical aspects of preventive medicine, this work has provided, through the study of the three dimensional structure of antigens composed of sialic acid, an explanation of the unique shape that these antigens can adopt as components of complex cell surface molecules. These observations have far reaching implications in the field of cellular recognition.

Research on antibody structure and molecular recognition has revealed the crucial molecular features that direct the specificity of three monoclonal antibodies. The crystal structure of one Fab fragment has been solved and a second is under investigation. In the latter case a synthetic gene coding for the murine λ chain was successfully cloned and expressed in *E. coli*.

Studies of enteric diseases caused by *Salmonella*, *E. coli*, and *E. hermannii* have uncovered important new antigens some of which, in combination with the corresponding monoclonal antibodies, are of particular value for the rapid detection of as few as ten bacteria per gram of contaminated food sample.

Significant advances in the area of veterinary diseases have been made over the last four years. We have solved the structure of eight capsular and five cell wall lipopolysaccharide antigens of the major serotypes of *Haemophilus pleuropneumoniae*, an organism responsible for loss of swine in Canada through pleuropneumonia. Our work has provided the basis for improved diagnosis, epidemiology and possibly protection against this economically significant disease. Other ongoing studies involve the antigens of *Pasteurella haemolytica* an organism implicated in shipping fever of cattle, and of *Rhodococcus* species, which cause loss of foals due to respiratory infections.

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Ann. Inst. Pasteur/Microbiol., 139: 421-433, 1988.

Microbial Process
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G. Seifert
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Summer Students:
M. Forgie, E. LaCroix

The objective of the Microbial Processes Group is the enhancement of microbial processes using chemical, biochemical and engineering technologies. The main areas of interest are: anaerobic treatment of wastewaters from industrial and agricultural wastes; production of biologically active compounds using both free and immobilized cells; enhancement of enzymatic activities of microbial cells using chemical additives; and the development of new bioreactors for the simultaneous production and recovery of volatile compounds.

Research on wastewater treatment has focussed on the development and study of advanced anaerobic reactors. The reactor configurations include the downflow stationary fixed film (DSFF); the upflow blanket filter (UBF); the upflow anaerobic sludge blanket (UASB); and the anaerobic sequencing batch reactor. Particular emphasis is being given to the use of these reactors for treatment of priority pollutants, such as chlorinated phenolics and wastewaters from the pulp and paper industries. This major research effort is being carried out in collaboration with researchers at the Biotechnology Research Institute in Montreal, in industry and in universities.

Immobilization technologies are being studied for use with various biological systems. These include development and testing of both whole cell and cell-free systems for bioconversions as well as for the production of biologically active products. Parts of these projects are being carried out in collaboration with Canadian companies through a shared-cost agreement (IRAP). Additional efforts have focussed on the development of artificial cell systems.

In collaboration with the Microbial Products Group, there has been the development of a broad spectrum microbial oxidation technology for production of flavours and fragrances. Chemical and enzymatic manipulations of whole *Pichia pastoris* cells have resulted in the production of otherwise inaccessible oxidation products. These latter developments have resulted in the technology being submitted to CPDL for patent evaluation.

Studies on bioreactor development have focussed primarily on the design and testing of a computer-coupled high-rate gas recycle bioreactor in which the bio-oxidation products are removed from the unit. Successful testing has resulted in patenting action being taken. These studies have been combined with the development of an automatic aseptic bioreactor sampling system as well as software for both control and analysis of bioconversion and fermentation processes.

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Duff, S.J.B. and Murray, W.D.
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Matteau, P.P. and Seifert, G.K.E.
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Murray, W.D., Duff, S.J.B., Lanthier, P.H., Armstrong, D.W., Welsh, F.W. and Williams, R.E.
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Patent
Calleja, G.B., Lusena, C.V., Veliky, I.A. and Moranelli, F.
Conversion of carbohydrates to alcohol with certain yeasts.
Application No. 44,565; Filed 840314. Canadian Patent No.: 1,238,592; Issued 28 June 1988.

Microbial Products
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A. Brown
Summmer Students:
K. Dawson, L. Hamilton, K. Symanyk

The aim of the Microbial Products Group is to develop the industrial potential of microorganisms through studies of their physiology. We have identified two groups of microorganisms with important applications in biotechnology. The first are yeasts capable of producing natural flavour or fragrance compounds, and the second are the methanogenic bacteria essential in anaerobic waste treatment processes.

Increasing commercial interest in the production of food flavours by microorganisms is based partly on the ability to label such products as "natural". Two processes using yeast cells have been developed and assessed for the biogeneration of acetaldehyde; one has the capability to generate other aldehydes as well. Further work on flavour production involves "non aqueous" esterification and lipase mediated transformations. Various food grade oils have been investigated to serve either as reaction media or as extractants of desired products from aqueous solution.

Several key genera of methanogenic bacteria have been isolated, including a slow growing methanogen capable of growth and methane production from acetate. Knowledge acquired through nutritional studies of these isolates has been applied to a waste disposal problem of a leading pulp and paper mill. The process was demonstrated on site at the 51,000 liter scale, and has lead to a successful full-scale field trial.

Detailed studies have been conducted on a number of unique chemicals and enzymatic reactions found in the cells of methanogens. These include novel lipids, sheath structures, and metal binding capacities. ^{13}C-nuclear magnetic resonance methods revealed metabolic pathways well suited to the production of specifically labelled compounds.

In addition to the major research themes, a system for direct labelling of DNA has been completed, and a new animal cell bioreactor has been designed for testing.

Microbiological expertise is provided to the Division via the maintenance of a comprehensive microbial culture collection and fermentation facility.

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Archives of Microbiology, 150: 338-342, 1988.

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Biotechnol. Bioeng. 31: 790-795, 1988.

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Patents

Armstrong, D.W., Martin, S.M. and Yamazaki, H
Selective production of ethyl acetate and acetaldehyde by microorganisms.
U.S. Patent #4,720,457.

Polysaccharide Processing
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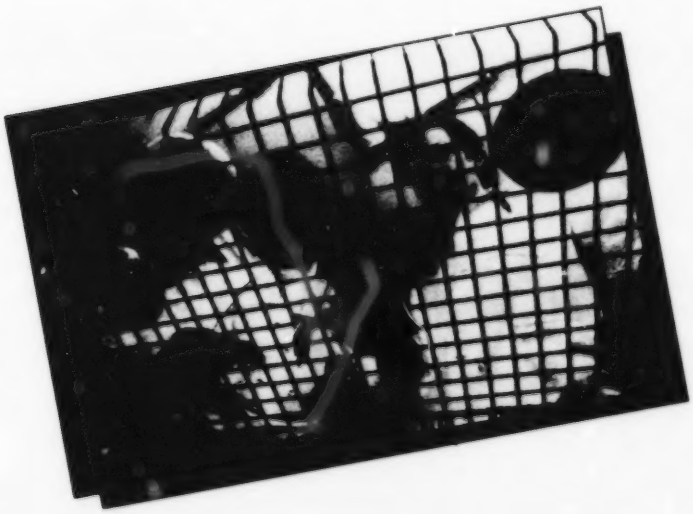
Summer Students:
C. Braun, E. Seguin, A. Vyse

The general objective of the Polysaccharide Processing Group is to improve commercial and agricultural utilization of polysaccharides of plant cell wall materials. More specifically, we are interested in developing enzymic methods to modify polysaccharides in a selective and controlled manner that would be of commercial interest. An example is the use of enzymes to modify pulp to increase water holding properties or to decrease the cost of bleaching. We are also interested in developing enzymic or other methods that enhance the rate and extent of hydrolysis of ligno-cellulosics, with a view to increasing their utility in bio-conversion processes of potential commercial use.



The structures of plant cell wall polysaccharides are complex and a variety of different enzymes are required for their hydrolysis. An approach used to enhance hydrolysis centers on the identification of enzymic steps that control or limit rates. One such step is the removal of substituents on the main chain of xylans. Enzymes that remove a number of different substituents have been fractionated, with the intention of evaluating their role in controlling hydrolysis rates and then devising suitable strategies for overcoming any rate limitations present. Xylanases have been fractionated as well with a view to evaluating their role in the hydrolytic process, since they can act synergistically with substituent-removing enzymes. These xylanases are also of interest in modifying the properties of pulp.

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Can. J. Microbiol. 34: 787-772, 1988.

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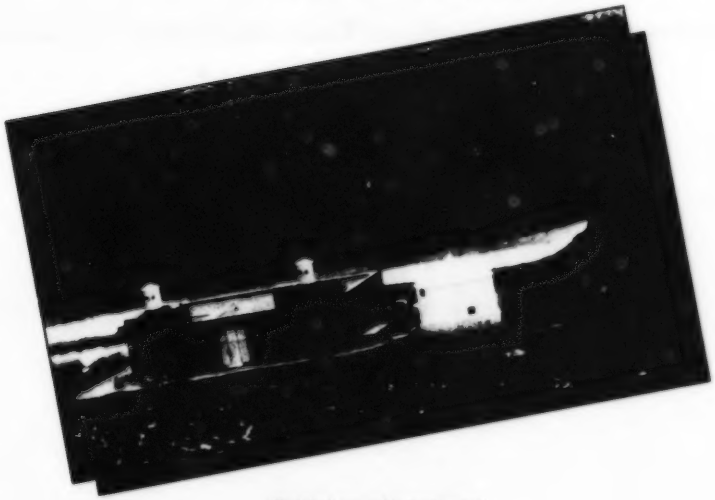


Municipal anaerobic treatment of leachate from landfill site, developed in collaboration with the Microbial Products Group. (Courtesy of Porter and Dillon, Halifax)

Administration and services

Administrative Officer:
D.A. Townsend

Office:
A. Daley
E. Monson
C.J. Gobey
L.M. Boucher
J.A. Strang
J.A. Legari
K.I. Hamelin

Mechanical Shops:
J.P. Dumont
J.S. Lynn
H.G. Lippke
A.T. Trudel
Y.E. Leveille
P.W. McFadden

Photography:
T. Devecseri
H. Turner

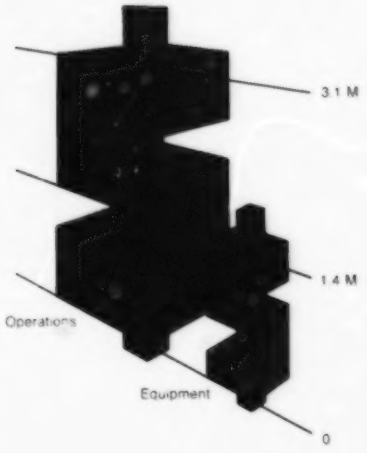
Electronic Shops:
G. Charette
D.E. Harris
R.B. Holmes
W.P. Kasperek
R.J. Mausser
R.B. Toews
A. Simard
R.J. Athey

Supply:
E.E. Grosser
S.G. Belec
T.C. Murphy
P.J. Ashley
S.H. Fletcher

The NRC full-time staff target for the Division in 1988/89 was 179 people. In addition, there were many guest scientists and students collaborating and working in the laboratories, many on a part-time basis:

Divisional Machine Shop.

The NRC supplied budget in the 1988/89 fiscal year for the Division, other than for salaries, was:



The following graph provides the distribution of personnel over the laboratories and services.